possibility of having a fast and highly processive method for dosing blood PMP levels is essential to adjust the dose during antiepileptic multi-drug therapy. Although mass spectrometry (MS) coupled to liquid chromatography remains the gold-standard to quantify this kind of molecules in biological matrices, the availability of a HPLC-based method allows to obtain a reliable dosing of this drug in clinical settings where MS is not available.

DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR OROTIC ACID AND OROTIDINE 5'-MONOPHOSPHATE DETECTION IN HUMAN PLASMA AND URINE

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BACKGROUND: The orotic acid (2,4-dioxo-1H-pyrimidine-6-carboxylic acid; Vitamin B13) is an intermediate metabolite of pyrimidine nucleotides biosynthesis and represents a minor diet constituent. The precursors of orotic acid in human metabolism are the cytosolic carbamoyl phosphate and aspartate via dihydrorotate: this biosynthesis is catalyzed by CAD gene encoding multifunctional enzyme. The multimeric protein called uridine 5'-monophosphate synthase is constitued by two domains that catalyze uridine monophosphate synthesis: orotatephosphoribosyltransferase (OPRTase; EC 2.4.2.10) and orotidine 5'-phosphate decarboxylase (OMPdecase; EC:4.1.1.23). The complete pathways of orotic acid biosynthesis is reported in Fig. 1. The step (5) represented in Fig.1 is directly involved in metabolism of 5-Fluorouracil (5-FU), because this anticancer drug is competitive substrate of OPRTase. In particular, the transferase activity of OPRTase multicomplex enzyme is inhibited by 5-FU at 59% level of control. The other end OPRTase is involved in metabolic disorders as congenital orotic aciduria and consequently the urinary orotic acid is quantified in clinical routine analysis for differential diagnosis of hereditary metabolic disease. Therefore, we aimed to develop a high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) assay that allows the simultaneous and sensitive detection of an orotic acid and orotidine 5'- monophosphate as metabolic and toxicological biomarkers in plasma and urine.
METHODS: The implementation and validation of chromatography and spectrometric method are developed in accordance with UNI EN ISO/IEC 17025:2018 and Eurachem Guide Lines (Method validation in clinical chemistry follows the established standards and procedures accepted by all disciplines of chemical metrolo-

gy). The clinical aspects are tested by analyzing diagnostic proficiency testing (external quality assessment - EQA) and other samples from patients with metabolic and malignant disorders.
RESULTS: The analytes, orotic acid and orotidine-5'-monophosphate are identified and quantified with high performance parameters of repeatability, reproducibility, robustness, precision and accuracy. The quantification method is based on internal standard approach for signal and matrix effect suppression. Whatever analytes is identified and quantified by two MRM transition with S/N>50 in LOD range. The analytical method clearly distinguish between urine and plasma specimens from the normal and pathological patients at 97.5% of level of confidence.
CONCLUSIONS: The HPLC-MS/MS method to be suitable for differential diagnosis of hereditary metabolic disease and metabolic monitoring of toxicity induced by anticancer drug. The analytical protocol is rapid and ideal to be used in routine analysis of clinical chemistry laboratory.

EFFECT OF SEASONS ON FOUR ANTI-EPILEPTIC DRUGS PLASMA LEVELS

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BACKGROUND: Therapeutic drug monitoring of anti-epileptic drugs is widely use for the management of epilepsy and to avoid treatment failure or explain adverse events onset. In this study, weexploredthe role of months and seasons of withdrawal for plasma quantification of oxcarbazepine, lamotrigine, phenytoin and levetiracetam pharmacokinetics and outcome cutoffs prediction.
METHODS: One hundred and seventy-five adult patients were enrolled. Drugs plasma concentrationswere measured by HPLC-UV methods.
RESULTS: We reported that oxcarbamazepine levels were higher in autumn and winter than those reported in spring and summer. In logistic regression model, warm seasons have been retained as therapeutic range negative predictive factors.If we separately evaluate males and females, the influence of seasons on oxcarbamazepine concentration remained only in male patients, also considering the logistic regression analysis.No factors significantly influenced lamotrigine, phenytoin and levetiracetam levels or were retained in regression model as treatment outcomes predictive factors.
CONCLUSIONS: These results, for the first time, suggest the effect of seasons on oxcarbamazepine. Apply a seasonal and gender specific approach should be the key to optimize treatment in each patient, in each period of people life.